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Attempts to Determine the Community Structure of Archaea in Major Tributaries of the Mississippi River

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ATTEMPTS TO DETERMINE THE COMMUNITY STRUCTURE OF ARCHAEA IN MAJOR TRIBUTARIES OF THE MISSISSIPPI RIVER

By
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ABSTRACT

Archaea were once thought to be limited to extreme environments, but it has now been established that they are found in diverse ecosystems worldwide. Archaea may possess distinctive properties that affect biogeochemical processes, which makes understanding their distribution crucial in determining their effect on these processes. Little research has been done on the ecology of Archaea in rivers, and this study represents the first attempt to gain an understanding of the diversity of Archaea within major tributaries of the Mississippi River. Polymerase Chain Reaction (PCR) amplification of archaeal DNA and denaturing gradient gel electrophoresis (DGGE) was used to examine community structure, and DNA sequencing of specific DGGE bands was performed to identify these communities' component populations. Despite the use of archaeal specific PCR primers, no Archaea were recovered from the samples; although, various bacteria and one eukaryote were identified in the sequencing data. However, relationships were clear among the samples, with particle-associated communities being distinct from those that were free-living. These findings may have implications of future studies of Archaeal diversity within rivers, particularly regarding primer specificity.

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LIST OF ABBREVIATIONS

SSU	small subunit
rRNA	ribosomal ribonucleic acid
rDNA	ribosomal deoxyribonucleic acid
PCR	polymerase chain reaction
DGGE	denaturing gradient gel electrophoresis
Ark	Arkansas River
Ohi	Ohio River
Miz	Missouri River
Ten	Tennessee River
UpM	Upper Mississippi River
bp	base pairs
S	Svedburg
°C	degree Celsius
g	gram
mL	milliliter
SB	sodium borate
µL	microliter
V	volts
min	minute
TEMED	N,N,N',N'-tetramethylenediamine
H_2O	water
BLAST	Basic Local Alignment Search Tool

INTRODUCTION

The Mississippi River is the third longest river in North America and fourth longest river in the world. It flows from Lake Itasca all the way to where it empties out into the Gulf of Mexico. Not only is it crucial for agricultural purposes and for allowing humans to ship throughout the country, it is also home to a great diversity of species and habitat. The Mississippi River houses numerous species of all sorts of animals, including fish and reptiles. Its tributaries extend across the United States touching or going through 31 states (National Park Service 2014). The Mississippi River also likely houses a much greater amount of microbial species that have remained largely uninvestigated.

Compared to other ecosystems, there have been relatively few studies on the microbial ecology of rivers, despite the accepted importance of microorganisms to river function. Various studies have described theoretical models that predict changes in biological assemblages in river networks (Vannote et al. 1980; Junk et al. 1989; Thorp and Delong 1994), however they only cover microbial ecology in a superficial manner. Considering that bacteria contribute greatly to the carbon cycle in riverine systems (Cole et al. 2007), adopting a more detailed microbial perspective could assist these theoretical models, which could then help explain variation in the structure of microbial communities (Millar 2013). This project explores Archaeal biological diversity in the Mississippi River and its major tributaries.

Despite playing an important role in various ecosystems, Archaea were not discovered until the 1960's. Archaea make up the third domain of life, alongside Bacteria and Eukaryotes. The classification of Archaea as a unique evolutionary group was determined by universal small subunit ribosomal RNA (SSU rRNA) and protein phylogenetic trees (Woese et al. 1990). This basis for classification has since been verified by comparative genomics (Gribaldo and Brochier-Armanet 2006). Archaea were initially assumed to be located exclusively within extreme environments; however, it is currently known that Archaea are found in numerous environments both on land and in water. The unique properties of Archaea make understanding their community structure necessary in order to gain insight into earth's global processes. Some Archaea are able to perform methanogenesis while others can survive in extreme pH, temperature or salty environments. Archaea may also make a significant contribution in the biogeochemical processes of earth (Gribaldo and Brochier-Armanet 2006). In order to gain further insights into these mechanisms, one must investigate Archaea in a variety of ecosystems, including rivers.

The application of molecular techniques has aided researchers in studying Archaea more effectively. Much of the present knowledge of the community structure of microbial diversity stems from cultivation methods. The microbes that have been cultivated from traditional methods represent only a small portion of the prokaryotes on earth, and so it is possible that products from cultivation are not representative of the diversity in the sample (Bintrim et al. 1997). Using the sequencing techniques described by Woese and Fox (1977), researchers are now able to gain more specific insight into the community structure of Archaea (Millar 2013). For example, comprehensive information

on the microbial diversity of halophilic Archaea in hypersaline ecosystems has been acquired through the use of PCR amplification and sequencing of 16S rDNA genes, followed by comparison to rapidly growing public databases (Oren 2002).

Although the diversity of bacteria in freshwater has been studied relatively extensively, the diversity of Archaea has been largely unexplored in most aquatic systems, and the Mississippi River is no exception. The objective of this project was to investigate samples taken from different tributaries of the Mississippi River for the presence of Archaea, and if present, to characterize the structure of these archaeal communities. This was accomplished through PCR amplification of Archaeal DNA and use of denaturing gradient gel electrophoresis (DGGE) to examine community structure. DNA sequencing of specific DGGE bands was performed to identify these communities' component populations.

METHODS

Site selection and sampling

Samples were collected as part of a broader project looking at the distribution of bacterial communities in major tributaries of the Mississippi River (Millar 2013). Samples were collected from five major tributaries of the Lower Mississippi River, with three different sites sampled from each tributary. Sites were 50-100 km apart with the most downstream site being just above the confluence with the greater system. The tributaries involved in the broader sample collection were: the Arkansas River (Ark), Ohio River (Ohi), Missouri River (Miz), Tennessee River (Ten), and Upper Mississippi River (UpM). These samples were obtained from July 6 to 17, 2012. An alphabetical system was utilized to label the sites within the rivers, with the most upstream site labeled as "A" and letters increasing downstream. All samples within a river were obtained on the same day between 8:00 am and 2:00 pm, starting from the lowest point downstream and working upstream. Samples were taken from mid-channel at a depth of approximately 0.5 m (Millar 2013).

For this study, samples came from the Upper Mississippi, Arkansas, Missouri and Ohio rivers. These samples were chosen as they showed good yields of bacterial DNA, suggesting that Archaea might also be present. Three samples came from different sites on the Upper Mississippi, one sample from the Arkansas, two samples

from the Missouri, and one sample from the Ohio. Samples from the Upper Mississippi and Ohio rivers were associated with suspended particles>3 microns in diameter, while the sample from the Arkansas River represented free-living cells (i.e. it was from water that passed through a 3 micron filter). The two samples from the Missouri were one particle-associated and one free-living. Details of sample processing and filtering are given by Millar (2013).

DNA extraction and amplification

DNA was obtained from the samples through the use of a PowerWater DNA Isolation Kit (MoBio, Carlsbad, CA), following the manufacturer's instruction. Archaea specific primers were used to amplify the DNA via the Polymerase Chain Reaction (PCR). The primers initially used for PCR were Arc2f (5'-TTCCGGTTGATCCYGCCGGA-3') and Univ1492r (5'-GGTTACCTTGT TACGACTT-3'). Multiple rounds of amplifications were performed using these primers, and they consistently yielded negative results. Therefore, a different set of primers was used. The new primers used for PCR were Arc931f (5'-AGGAATTGGCGGGGGAGCA-3') and Univ1392GC (5'-ACGGGCGGTGTGTGC-3'). Two cycles of amplification using these primers were performed on the samples. These primers amplify a 461 bp region of the 16S rRNA gene, presumably just from the Archaea given the specificity of the Arc931f primer. Primers were those used by Jackson et al. (2001) based on sequences reported by Amann et al. (1995). The PCR process consisted of three steps over a range of 40°C and a duration of 13 minutes. The denaturing step was performed at 95°C for 2 minutes. Then 23 cycles were performed at

95°C, 45°C, and 72°C for a duration of 1 minute, 1 minute, and 2 minutes respectively. Finally, an elongation step was performed at 72°C for 7 minutes (Millar 2013). PCR products were subsequently viewed by agarose gel electrophoresis, using gels prepared with 1.5g of agarose and 100mL of sodium borate (SB) buffer. Next, 5 µL of sample and 1.5 µL of dye were loaded into the gels, which were electrophoresed for 25 min at 220 V.

Denaturing gradient gel electrophoresis

Denaturing gradient gel electrophoresis (DGGE) was used to examine the diversity of sequences in PCR products and to compare archaeal community structure between samples. This process denatures the DNA differentially based on its melting properties, essentially separating a mixed set of PCR products into fragments from individual populations. A DGGE-2001 system was used for this process (C.B.S. Scientific Co., Del Mar, California) using a gel with a 40-70% gradient of the denaturants formamide and urea in 4% acrylamide. The polymerizing reagents used were 10% ammonium persulfate and N,N,N',N'-tetramethylenediamine (TEMED). The DGGE gel was run overnight for 19 hours at 83 V, and subsequently stained in Sybr Green and viewed under UV-transillumination. After images were taken, selected individual DGGE bands were then cut from the gel. The bands were cut with an X-tracta Gel Extractor tool (Promega Corporation, Madison, WI). Brighter bands on the gel were specifically targeted for excision, as they were more likely to yield valid DNA. Excised gel fragments were transferred to individual tubes and stored with 10 µL sterile H_2O at -20°C. Next, 2 µL of the fragment water was used as the template in subsequent PCR amplifications to re-amplify the gel band, using the same conditions reported earlier.

Amplifications were viewed on agarose gels, and 19 of the excised bands appeared to show the correct fragment after PCR. These 19 samples were sent out for sequencing at a dedicated facility (Functional Biosciences Inc., Madison, WI), and 11 valid sequences were obtained.

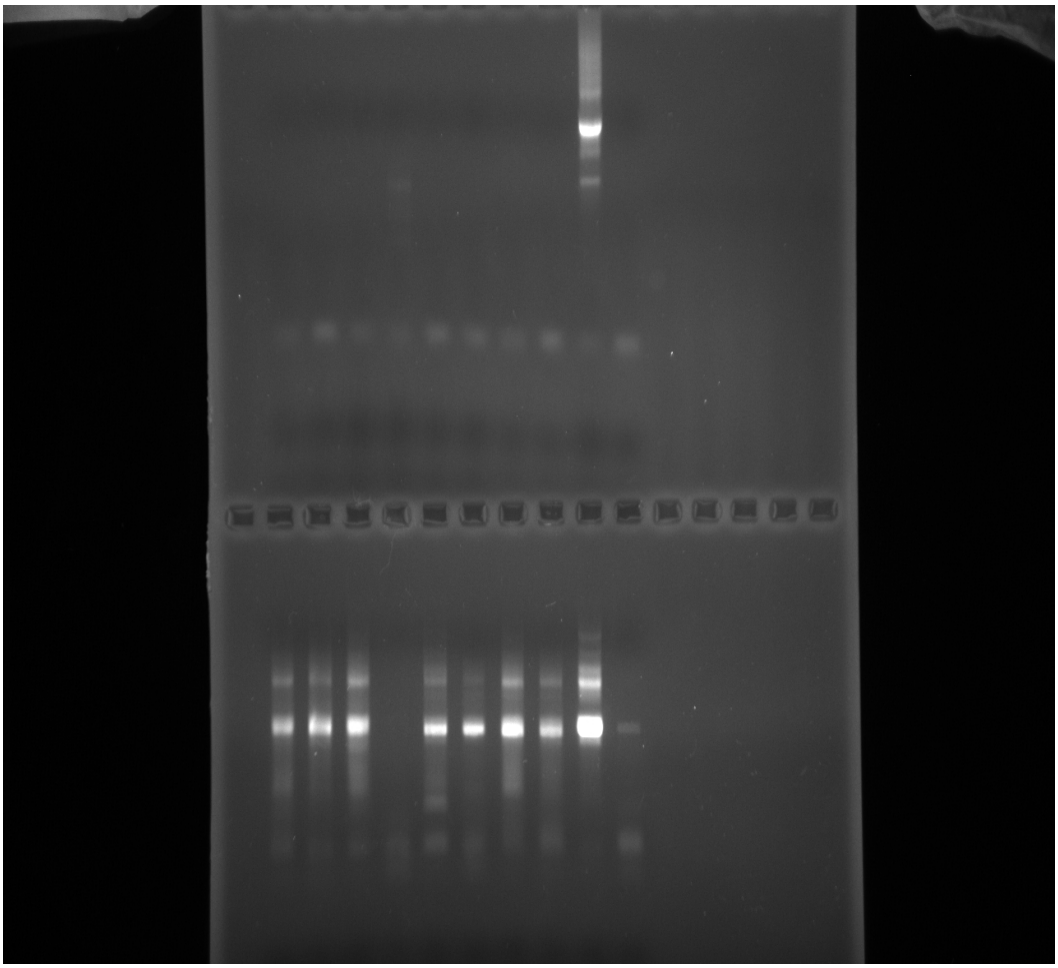
Data analysis

Banding patterns on the DGGE gel were converted into binary data indicating the presence (1) or absence (0) of a specifically migrating band in each sample. Binary data was then used to create a measure of similarity (Jaccard index) between samples, using the software Mothur (Schloss et al. 2009). Similarity scores were used to generate a dendrogram relating community structure in each sample (as derived from DGGE banding patterns) to each other. Sequences derived from the 11 valid excised DGGE bands were entered into a nucleotide BLAST search of the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the three closest matches noted.

RESULTS

Archaeal PCR and DGGE Analysis

Initial amplifications using the Archaeal primers Arc2f and Univ1492r yielded negative results. Products were eventually generated following multiple rounds of amplification using the primers Arc931f and Univ1492r. Two cycles of amplification were required to obtain results. This process yielded PCR products from most samples (Fig. 1) with the exception of Ark-C-6, which was therefore excluded from DGGE analysis.



1 2 3 4 5 6 7 8 9 10

Fig. 1. Gel resulting from multiple rounds of amplification. The top half of the gel is from one round of amplification using the primers Arc2f and Univ1492r followed by another round of amplification using the primers Arc931f and Univ1392GC. The bottom half of the gel is from two rounds of amplification using the primers Arc931f and Univ 1392GC. Samples beginning with lane 1: UpM-A-46, UpM-A-57, UpM-C-1, Ark-C-6, Ark-A-40, Miz-B-92, Miz-B-51, and Ohi-B-5.

DGGE separated the amplified DNA in each sample into component populations, as represented by individual bands on the DGGE gel. The number of bands in each sample ranged from 2-13 with a mean of 5 bands per sample. In the seven lanes of samples on the DGGE gel, Miz-B-92 contained the most bands with 13, and UpM-B-57 contained the second most bands with six. UpM-A-46 and Ark-A-40 both contained four bands, while UpM-C-1 contained three bands and Miz-B-51 and Ohi-B-5 each contained two bands (Fig. 2).

These data was used to create a dendrogram to demonstrate the relationships among the samples (Fig. 3). The Upper Mississippi river samples were all particle-associated and contained similar populations, with UpM-C-1 and UpM-A-46 being the most similar samples. UpM-B-57 is closely related to the other two Upper Mississippi samples. Ohi-B-5 and Miz-B-51 were similar to each other and formed a distinct group from the other samples, both of which were particle-associated. Miz-B-51 and Miz-B-92, which were particle-associated and free-living respectively, seemed to contain distantly related populations. Ark-A-40 formed its own distinct group; however, it seemed to be distantly related to Miz-B-92, both of which are free-living.

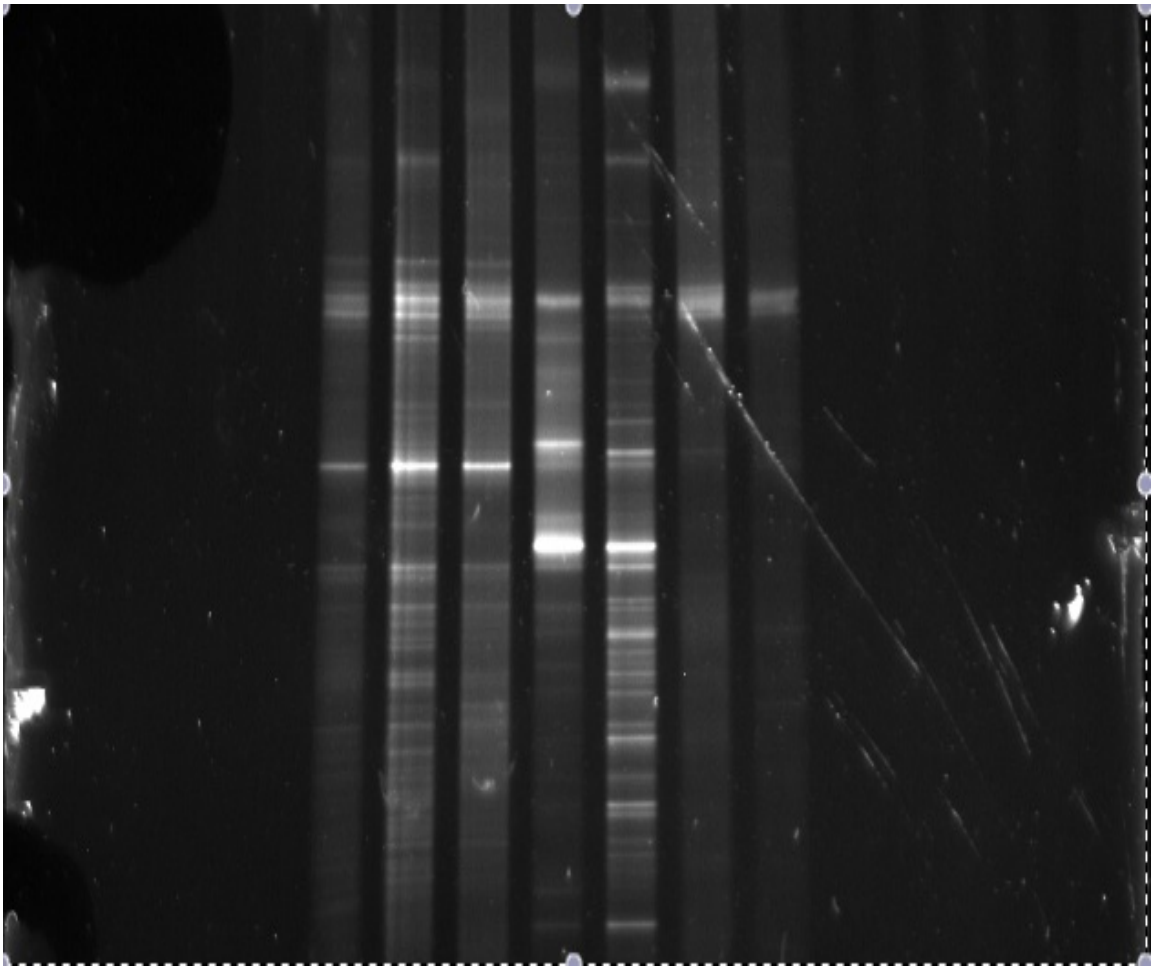


Fig. 2. DGGE showing the component populations of the seven samples that were obtained from different tributaries of the Mississippi River. Samples from left to right: UpM-A-46, UpM-B-57, UpM-C-1, Ark-A-40, Miz-B-92, Miz-B-51, and Ohi-B-5.

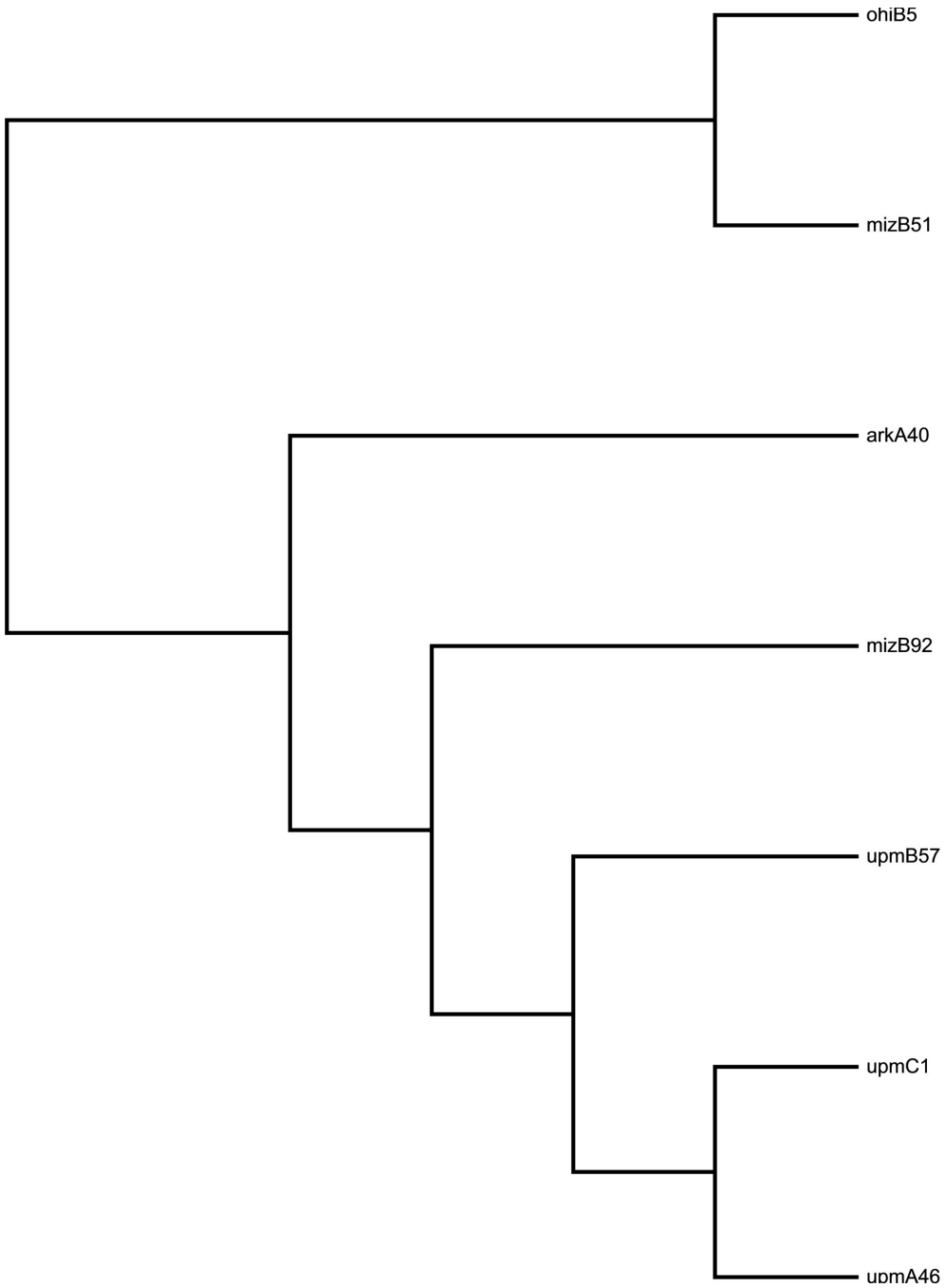


Fig. 3. Dendrogram showing the relationships between DGGE profiles based on banding patterns converted into binary data.

DNA Sequencing

A total of 19 bands were successfully excised and recovered from the DGGE gel: 10 from the Upper Mississippi samples and nine from the Missouri (Fig. 4,5). Of these, 11 were successfully sequenced. However, following a BLAST search, all 11 sequences were identified as being bacterial or eukaryotic in origin rather than archaeal. Three bands were identified as *Salpingoeca*, a eukaryote, belonging to the group choanoflagellates, all of which were obtained from the Upper Mississippi River samples. Miz-B-20 was identified as *Algoriphagus*, which is a genus of bacteria belonging to the phylum *Bacteroidetes*, and Miz-B-23 was identified as an uncultured planctomycete clone. The remaining bands were found to be various uncultured bacteria (Table 1).

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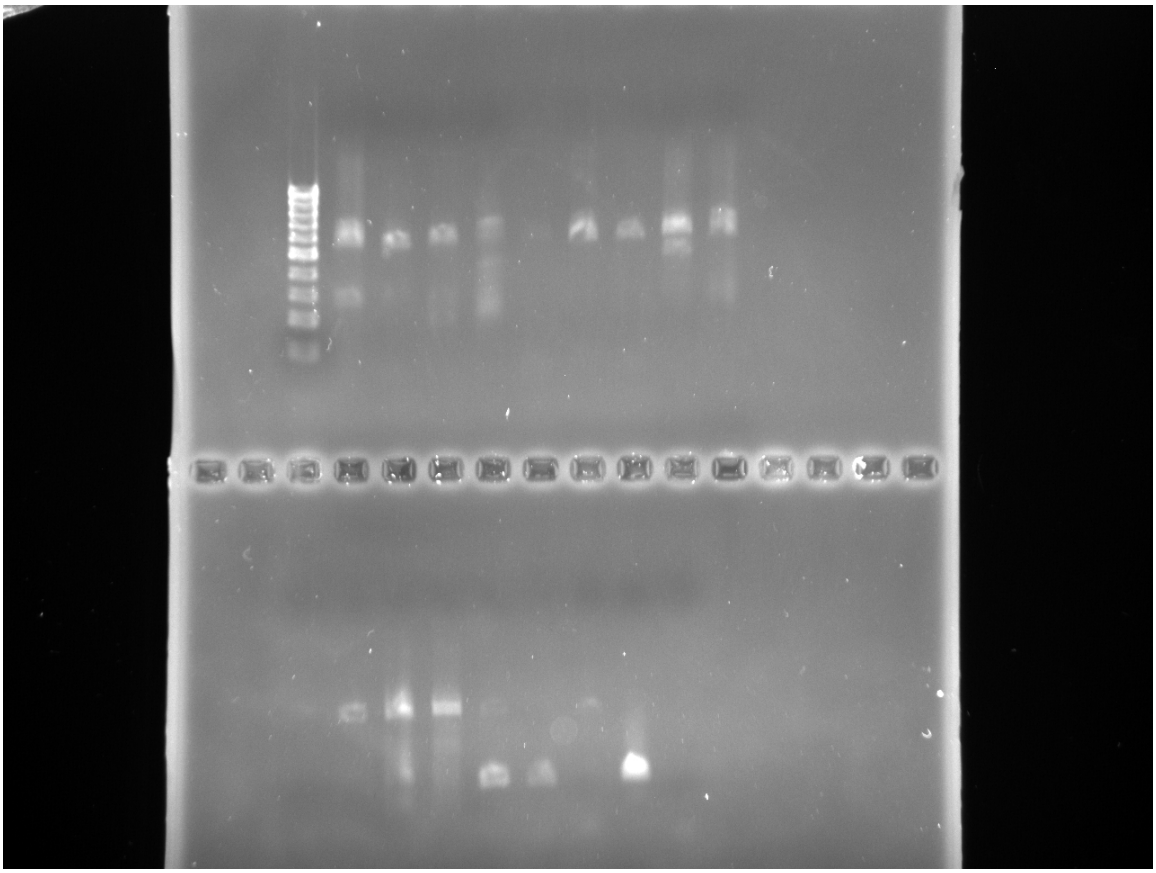

Fig. 4. Gel resulting from PCR of the DNA recovered from the first half of extracted DGGE bands. Samples starting from top of gel moving left to right: four bands from UpM-A-46, six bands from UpM-B-57, three bands from UpM-C-1, and four bands from Ark-A-40. DNA ladder was erroneously excluded from the bottom half of the gel.

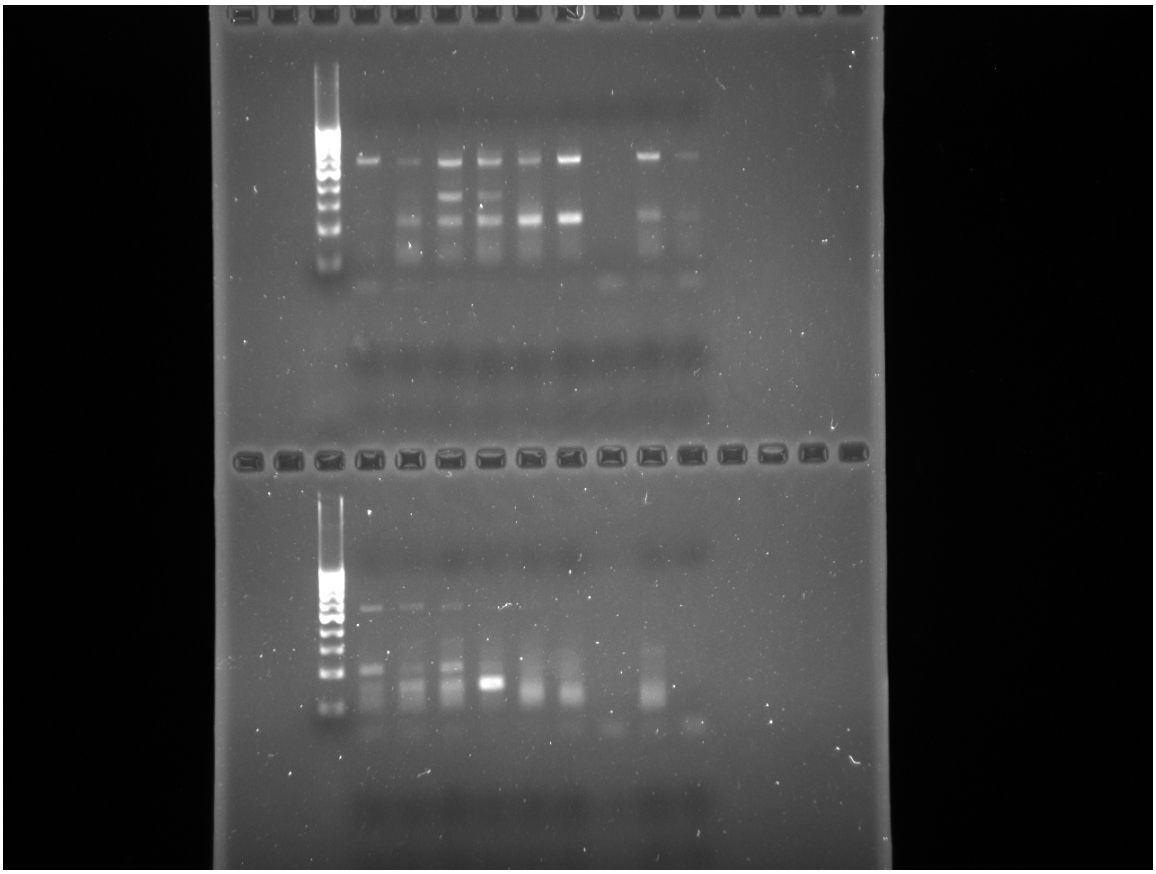

Fig. 5. Gel resulting from PCR of the DNA recovered from the second half of extracted DGGE bands. Samples starting from top of gel moving left to right: thirteen bands from Miz-B-92, two bands from Miz-B-51, and two bands from Ohi-B-5.

Table 1. Identities of dominant bands in DGGE gels of PCR products produced with archaeal specific primers from samples from the Upper Mississippi (UpM) and Missouri (Miz) rivers.

Sample	Closest Match	% match	Accession # for 3 closest matches
Miz-B-20	*Algoriphagus boseongenesis* strain BS-R1	99% 99% 99%	KF768344.1 HG529990.1 AY264838.2
Miz-B-23	Uncultured planctomycete clone from lakes in northeastern Germany	99% 99% 99%	DQ501296.1 AF428856.1 AF428892.1
Miz-B-25	Uncultured bacterium gene for 16S rRNA from rivers in Malaysia	99% 99% 99%	AB698042.1 EU803846.1 AB753977.1
UpM-A-2	Uncultured bacterium clone C-102 from the streams of Dianchi Lake, China	99% 99% 99%	HQ860578.1 FJ612438.1 FJ612294.1
UpM-A-3	*Salpingoeca* sp. ATCC 50818	97% 93% 87%	XM_004997410.1 FO818640.1 CP007156.1
UpM-B-6	Uncultured bacterium clone SanDiego_16467 from the gut of animals	99% 99% 99%	KF799706.1 HQ226289.1 HQ226233.1
UpM-B-7	Uncultured bacterium clone SW-Oct-48 from raw seawater	99% 99% 99%	HQ203768.1 KF799706.1 JX864480.1
UpM-B-8	*Salpingoeca* sp. ATCC 50818	97% 100% 93%	XM_004997410.1 XM_002037777.1 FO818640.1
UpM-C-11	Uncultured bacterium clone 39_135 from the Gulf of Gdansk	99% 99% 99%	KF596535.1 JN207204.1 KC425548.1
UpM-C-12	Uncultured bacterium clone C-68 from Dianchi Lake, China	100% 100% 100%	HQ860601.1 HQ860578.1 FJ612438.1
UpM-C-13	*Salpingoeca* sp. ATCC 50818	97% 93% 87%	XM_004997410.1 FO818640.1 CP007156.1

DISCUSSION

Few studies have examined the diversity of Archaea in rivers, and none have attempted this in the Mississippi River. The diversity of Archaea was examined in the Mackenzie River, which flows into the arctic seas, and the Beaufort Sea (Galand et al. 2006). That study found a different composition of Archaea at each site and a large amount of diversity. In a study of the microbial diversity of Archaea in the Tinto River, DGGE analysis revealed various groups of Archaea, but amplification with rRNA-targeted oligonucleotide probes showed that Archaea accounted for only a small percentage of the microbial community (González-Toril et al. 2003). The goal of this project was to use similar approaches to these studies to attempt to gain insight into the ecology of Archaea within various tributaries of the Mississippi River. However, multiple rounds of amplification through archaeal specific PCR and subsequent analysis by DGGE and 16S rRNA sequencing of DGGE bands failed to yield any sequences that could be confirmed as Archaea. Even though this does not disprove the existence of Archaea in these systems, their presence seems improbable since Archaea were consistently absent in the sequences examined. One possible explanation could be that there may have been much larger numbers of bacteria in the water samples. These bacteria could have overpowered the archaeal DNA during PCR so that only bacterial DNA was amplified to useable levels.

Even though the sequenced bands did not correspond to Archaea, the DGGE profiles still provide some measure of community similarity between samples. All of the Upper Mississippi river samples showed similar community profiles, and they were all particle-associated. This pattern appears to hold true for the remaining samples; e.g. Ohi-B-5 and Miz-B-51 were similar to each other and form a distinct group from the other samples, and they were both particle-associated. This reinforces the idea that there is a difference between particle-associated and free-living microbial communities, a pattern that was evident for bacterial assemblages in these systems (Millar 2013). The two samples collected from the same tributary (Miz-B-51 and Miz-B-92) further demonstrate this finding, as they represent a particle-associated and free-living sample respectively, and contained only distantly related populations according to DGGE profiles. Even though Ark-A-40 formed its own distinct group, it does seem to be somewhat similar to Miz-B-92, and both are free-living. Thus, the sample type (particle or free-living) appears to be more important than river of origin in terms of community similarity. A relationship between free-living and particles and microbial structure has been described by Kellogg and Deming (2009). These researchers also found that the size of each class of particle generally determined the species richness of Archaea, with the smaller sized particles containing more archaeal diversity and the larger particles containing lower archaeal diversity.

While no Archaea were identified in the DGGE bands that were sequenced, various bacteria and one eukaryote were detected. UpM-A-3, UpM-B-8, and UpM-C-13 all contained *Salpingoeca* sp. ATCC 50818, now known as *Salpingoeca rosetta*. *S. rosetta* is a eukaryote, belonging to the group choanoflagellates. This species inhabits

various habitats, and it has been recovered from mud near Hog Island, Virginia (Alegado et al. 2013). Although it appears that *S. rosetta* inhabits a variety of environments, there are no reports of their isolation from rivers, so their discovery in this system may be a novel finding. A band from Miz-B-20 was identified as *Algoriphagus boseongenesis*, a species of bacteria, belonging to the phylum Bacteroidetes, which has been previously recovered from a tidal flat (Park et al. 2013). As with *S. rosetta*, no sources were found that indicated this species inhabits a river.

For one of the bands obtained from Miz-B-23, the second closest match is to a bacterial sequence obtained from the Changjiang River (Sekiguchi et al. 2002), a large river in China. Even though this match is not to Archaea, it is interesting that this same species of bacteria that was found in two large rivers on different continents, and suggests that it may represent a species adapted to such ecosystems. Similarly, a band sequenced from Miz-B-25 matched to an uncultured bacterial 16S rRNA gene that was obtained from rivers in Malaysia (Hidayat et al. 2012), reinforcing the idea that some microbial populations may be adapted to riverine environments and found throughout the world. Other matches supporting this idea include a band from UpM-A-2 that matched a 16S rRNA gene sequence obtained from the streams of Dianchi Lake in China (Lv and Huang 2012), and a sequence obtained from UpM-C-12 matched to a similar, yet different cloned sequence from the same study.

A few sequences were more related to those previously obtained from marine samples, as the closest match to a sequence from UpM-B-7 was a gene sequence recovered by Bae and Lee (2010) from raw seawater. A sequence from UpM-C-11 matched to a cloned 16S rRNA gene from the Gulf of Gdansk (Ameryk et al. 2013). A

particularly interesting match was a band obtained from the Upper Mississippi sample UpM-B-6, which showed the strongest match to an uncultured bacterium clone that was obtained from the gut of the sea squirt *Ciona intestinalis* (Dishaw et al. 2014).

Even though no Archaea were present in the sequencing data, some interesting relationships and patterns were observed among the samples, and these may serve as a basis for future research on the ecology of Archaea in the Mississippi River. An important issue is that regarding primer specificity, as the primers used included one that is regarded as archaeal specific, but in this case the only sequences identified belonged to bacteria and a eukaryote. This could be partly due to the low (~45°C) annealing temperature in the PCR profile. Regardless, the community patterns were apparent and whether the sample was obtained as a particle or free-living seemed to play a major role in determining the degree of relatedness among the samples, a pattern that has been reported for bacterial communities (Millar 2013). Archaea are clearly much more genetically and environmentally diverse than originally thought, and more research and methodological development is required to gain a truer understanding of the diversity of Archaea in natural environments.

LIST OF REFERENCES

Alegado RA, Grabenstatter JD, Zuzow R, Morris A, Huang SY, Summons RE, King N (2013). *Algoriphagus machipongonensis* sp. nov. co-isolated with a colonial choanoflagellate. Int J Syst Evol Microbiol 63: 163–168

Amann R, Ludwig W, Schleifer KH (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59: 143-169

Ameryk A, Hahnke R, Gromisz S, Kownacka J, Zalewski M, Szymanek L, Calkiewicz J, Dunalska J, Harder, J (2013). Bacterial communities in the Gulf of Gdansk. Unpublished manuscript.

Bae H, Lee S (2010). Microbial community structure in SWRO pre-treatment systems (raw seawater). Unpublished manuscript.

Bintrim SB, Donahue TJ, Handelsman J, Roberts GP, Goodman RM (1997). Molecular phylogeny of archaea from soil. Proc Natl Acad Sci USA 94: 277–282

Cole JJ, Prairie NF, Caraco NF, McDowell WH, Tranvik LJ, Striegl RG, Duarte CM, Kortelainen P, Downing JA, Middelburg JJ, Melack J (2007). Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10:171-184

Dishaw LJ, Flores-Torres J, Lax S, Gemayel K, Leigh B, et al. (2014). The gut of geographically disparate *Ciona intestinalis* harbors a core microbiota. PLoS ONE 9(4): e93386. doi:10.1371/journal.pone.0093386

Galand PG, Lovejoy C, Vincent FV (2006). Remarkably diverse and contrasting archaeal communities in a large arctic river and the coastal Arctic Ocean. Aquat Microb Ecol 44: 115–126

Gonzalez-Toril E, Llobet-Brossa E, Casamayor EO, Amann R, Amils R (2003). Microbial ecology of an extreme acidic environment, the Tinto River. Appl Environ Microbiol 69: 4853– 4865

Gribaldo S, Brochier-Armanet C (2006). The origin and evolution of Archaea: a state of the art. Philos Trans R Soc Lond B Biol Sci 361: 1007–10

Hidayat T, Samat MAA, Elias MAB, Hadibarata T (2012). Metagenomic analysis of 16S rRNA sequences from selected rivers in Johor Malaysia. Journal of Applied Sciences, 12: 354-361

Jackson CR, Langner HW, Donahoe-Christiansen J, Inskeep WP, McDermott TR (2001). Molecular analysis of microbial community structure in an arsenite-oxidizing acidic thermal spring. Environ Microb 3:532-542

Junk WJ, Bayley PB, Sparks RE (1989). The flood pulse in river-floodplain systems, .p 110-127 In DP Dodge [eds.] Proceedings of the International Large River Symposium, Can Spec Publ Fish Aquat Sci 106

Kellogg CTE, Deming JW (2009). Comparison of free-living, suspended particle, and aggregate-associated bacterial and archaeal communities in the Laptev Sea. Aquat Microb Ecol 57:1-18

Lv M, Huang Y (2012). Bacterioplankton community in the streams of Dianchi Lake, China. Unpublished manuscript.

Millar, JJ. (2013). Bacterial assemblages and microbial enzyme activity in the major tributaries of the Lower Mississippi River. MS thesis. University of Mississippi

"Mississippi River Facts." *National River & Recreation Area*. National Park Service, 15 April 2014

Oren, A (2002). Molecular ecology of extremely halophilic archaea and bacteria. FEMS Microbiol Ecol 39: 1–7

Park S, Park JM, Lee KC, Yoon JH (2013). Algoriphagus boseongensis sp. nov, a member of the family Cyclobacteriaceae isolated from a tidal flat. "Antonie Van Leeuwenhoek." 105(3): 523-531

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75: 7537–7541

Sekiguchi H, Watanabe M, Nakahara T, Xu B, Uchiyama H (2002). Succession of bacterial community structure along the Changjiang River determined by denaturing gradient gel electrophoresis and clone library analysis. Appl Environ Microbiol 68(10): 5142-5150

Thorp JH, Delong MD (1994). The riverine productivity model: an heuristic view of carbon sources and organic processing in large river systems. Oikos 70(2): 305-308

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980). The river continuum concept. Can J Fish Aquat 37: 130-137

Woese CR, Fox GE (1978). Phylogenetic structure of the prokaryotic domain the primary kingdoms. Proc Natl Acad Sci USA 74(11): 5088-5090

Woese, CR, Kandler O, Wheelis ML (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria and Eucarya. Proc Natl Acad Sci USA 87: 4576–4579